

ON A REMARKABLE BACTERIUM (*STREPTOCOCCUS*)
FROM WHEAT-ENSILAGE.

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(PLATE XII.)

A short time ago I obtained through the kindness of Mr. A. Bruce, Chief Inspector of Stock for New South Wales, some of the wheat-ensilage which had been used at Coonong, Urana District, N.S.W., as food for horses, amongst which a fatal epidemic, though of short duration, subsequently broke out. The samples under notice were of three descriptions: one was labelled as "fresh ensilage," another as "three or four days exposed showing mould fungus, as given to the horses," a third "fully developed mould fungus."

Among the micro-organisms—in all, three kinds of bacteria, and two kinds of moulds—which I cultivated out of the last-mentioned sample, there was one bacterial species that especially struck my fancy, and is interesting in more than one respect.

Starting with an infusion of the particular sample in a sterilised .6 p.c. common salt solution in a test-tube, and cultivating a minute part of it (or even a dilution of this minute part) in 10 p.c. nutritive gelatine on glass plates, for a few days, and at a temperature of about 20° C. (68° F.), one finds, at the surface of the layer of gelatine, amidst vegetations of the other micro-organisms, small greyish-white to slightly yellowish-white colonies (Pl. XII, fig. 1, x), having—at least the larger ones—their outlines irregularly crenate or emarginate. With the advancing enlargement of these aggregations or colonies, liquefaction of the gelatine underneath the latter commences, and the colonies themselves now present beautiful whitish substantial patches, the

central, greater part of which is watch-glass shaped, being situated in a watch-glass-like excavation, now filled with liquid gelatine (Pl. XII, fig. 2, a, b, c, d, e,); the periphery of the colonies forms a somewhat elevated zone or girdle round the inner mass, on the surface of not yet liquefied gelatine, and is made up, in rather an ornamental manner, of more or less elongated fringe-like processes or appendages, the arrangement of which is exhibited in Pl. XII, fig. 2, a-e.

On examination with a low power of the microscope (70-122 diam.) the contents of these colonies are seen to be crummy or flocculent. In the interior of the gelatine the micro-organism vegetates much more slowly and does not exhibit that beautiful arrangement of the superficial colonies.

I examined very young colonies, of from $\cdot 01$ — $\cdot 05$ mm. diam., and in their optical section they appeared, upon the whole, as circles or (not so often) as ellipses, whereas the older colonies have never been found of such a regular shape. But here and there small projecting or retrograding parts were met with in the contours which in themselves were not perfectly smooth and sharp, but looked as if lined with extremely minute teeth or prominences. The contents are finely granular, and of a more or less yellowish-grey colour (transmitted light).

On investigation with high powers of the microscope all these colonies are seen to consist of micrococci which occur singly, in twos, but more commonly forming strings or chains, often twisted or bent. (Pl. XII, fig. 3.) Hence the name *Streptococcus* for such kinds of micrococci. These chains are aggregated or grouped in clusters (which represent the substance of the colonies). The individual streptococci are more or less globular, and measure about $\cdot 0014$ in diameter. They stain intensely with methylene-blue solution, and other aniline dyes.

Inoculated into nutritive gelatine in a test-tube (pure cultivation) the *Streptococcus* grows along the course of the inoculating platinum wire to a slightly yellowish-white, somewhat flattened thread, made up at first of numbers of small beads. It is especially at the free surface of the gelatine that it propagates,

and here it commences to liquefy the latter in a funnel- or watch-glass-like manner, besides spreading itself beyond the margin of this excavation in the shape of a very thin, fragmentary, greyish-white film over the surface of the gelatine. Afterwards the growth in the gelatine presents an inverted conical bag filled with yellowish, densely packed flocky masses of the micro-organism. The liquefaction advances from above downwards, till, in course of time, there is seen in the test-tube one liquid mass, in which light, filamentous, fibrine-like masses (belonging to the micro-organism) are suspended, and at the bottom a noticeable deposit of yellowish colour.

On a sloping surface of a 1 p.c. nutrient agar-agar in a test-tube the organism multiplies readily at ordinary temperatures, and, after some days' standing, the cultivation presents a greyish, flat, superficial growth which appears wrinkled or folded. This is principally marked along the streak of inoculation, where the vegetation, being also more luxuriant, looks as if covered with short yellowish-white threads, interwoven with each other.

On the cut surface of a sterilised potato the *Streptococcus* likewise readily propagates. (Pl. XII, fig. 4.) After some time of incubation at ordinary temperatures, it develops to considerable masses of a peculiar yellowish-white tint and creamy consistency. These masses do not extend far over the nutrient surface. They resemble, following the track of the inoculating platinum wire, an elongated chain of mountains, the edges of which are characteristically emarginated, as if lined all along their contours with small, bead-like prominences. All over the surface of these plateaux, and quite close to one another, very shallow furrows are visible which run down to the borders of the masses; (conf. Pl. XII, fig. 4, where this appearance is roughly indicated.)

I may well note here that with the propagation of the *Streptococcus* a peculiarly sour but not very strong smell was associated, which was especially noticeable in the pure culture of the bacterium on a potato in a small glass capsule.

Whether this micrococcus, or the other Schizomycetes obtained from the wheat-ensilage in question, (Plate XII, fig. 1, x, y, z,) is pathogenic or not, has not yet been ascertained, as experiments on animals have not yet been tried. Unfortunately I am not in possession of preserved pieces of any of the organs of the horses which had been feeding on the ensilage, and afterwards succumbed to the reported epidemic. A microscopical examination of such organs would, undoubtedly, have proved a material aid in the elucidation of the question.

EXPLANATION OF PLATE XII.

- Fig. 1.—Part of a plate-cultivation in 10% nutritive gelatine of an infusion of the wheat-ensilage. The white colonies, x, belong to the *Streptococcus* described above; y, yellowish-green colonies of short bacilli liquefying the gelatine; z, bluish-white colonies of another bacillus.
- Fig. 2.—a, b, c, d, e. Some of the *Streptococcus*-colonies in a more advanced state of growth.
- Fig. 3.—a to l. Microscopical appearances of the *Streptococcus* (diagrammatic). The zones round the cocci represent the bright peripheral capsules observable in Bacteria.
- Fig. 4.—Cultivation of the *Streptococcus* on potato (p. 927).